MEASURING ROOT GROWTH RESPONSE TO NITROGEN FERTILIZATION RATES IN YOUNG PECAN SEEDLINGS USING THE MINIRHIZOTRON METHOD

A Thesis

by

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ABSTRACT

Pecan is an important nut crop in Texas and the United States. Increased demand for pecan exports has created an interest for new and current growers to plant new orchards. Nitrogen (N) application is an important step in establishing a productive pecan orchard, but few studies have determined how N affects the establishment of pecan tree roots, specifically fine roots (0-2 mm) responsible for nutrient uptake. The objectives of this study are to: 1) determine how fine root growth is impacted by five rates of N fertilizer and 2) pinpoint times in the growing season when fine root production peaks to time fertilizer applications for maximum N absorption.

The minirhizotron method was used to observe root growth through time at two soil depths, 14-28 cm and 98-112 cm, from February 2010 to June 2012. Images were collected every two weeks, roots were traced individually, and date of birth and death, diameter, and length were recorded.

Trees receiving 229.5 kg N ha^{-1} (1N) had the greatest standing root length throughout the study at both depths observed. The 2N treatment showed decreased standing root length compared to most other treatments. Two peaks in root growth were observed, in March 2010 and April 2011, when trees began to come out of dormancy. Living root length steadily declined throughout the rest of the growing season. The 1N treatment had greater cumulative root growth than the other treatments and there was a depth effect observed. Root lifespan was influenced by both N treatment and depth. Roots receiving higher rates of fertilizer (1N and 2N) and those living in 14-28 cm of

soil had a higher risk of mortality. Root birth season and diameter also affected lifespan. Roots born in spring and those with smaller diameters had a higher risk of mortality.

These results support current extension service recommendations that 229.5 kg N ha^{-1} is an appropriate amount of fertilizer and does not negatively affect root growth. However, application time could be moved to earlier in the season (March-April as opposed to May) to target the peak time for fine root growth and thus, N absorption.

DEDICATION

I would like to dedicate my thesis to my parents, Marc and Tammy. Thank you for your encouraging words, unconditional love, and for always believing in me.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Pecan [*Carya illinoinensis* (Wangenh.) K. Koch] is a native, deciduous tree of the Juglandaceae family (Moore, 2003). It has many uses including forage and habitat for wildlife, as well as timber for furniture and other wood products (Moore, 2003). It is also an important specialty nut crop in the United States. The United States produces between 200 and 300 million pounds of pecans each year of which Texas contributes about 60 million pounds, making it the third-largest producer after Georgia and New Mexico (National Agriculture Statistics Service, 2012). Increased demand for pecans, partially due to larger export to countries that have discovered the nut as a healthy snack, as well as the promotion of pecans as a health food in the United States, has led to more orchards being planted (M. Nesbitt, pers. comm.).

Nitrogen (N) application is among the most important management practices to ensure good pecan production (Smith *et al.*, 2007). Nitrogen is taken up by the plant and assimilated into amino acids that are important in translocation of organic N to nutrient sinks in the plant and act as N donors in many cellular reactions (Lam *et al.*, 1996). Increased pecan production is often associated with an increase in the amount of N fertilizer applied and introduced into the environment. The Texas Pecan Handbook, published by Texas A&M AgriLife Extension, recommends applying ammonium sulfate (21-0-0) the first year after tree transplant at 35 kg ha^{-1} in June if trees are growing

rapidly. Second-year trees should be fertilized with 200 kg ha^{-1} split into three applications in April, May, and June, and three-to-four year old trees with 207 kg ha^{-1} in April, May, and June (McEachern, 2012). Oklahoma Cooperative Extension recommends using a 10-10-10 fertilizer at the same N concentrations and at the same points during the growing season (McCraw *et al.*, 2012). According to Smith *et al.* (2000), the N application rates to use on pecan seedlings agree with those provided by both Texas A&M AgriLife Extension and Oklahoma Cooperative Extension for orchard establishment. Nitrogen deficiency can cause stunted growth and chlorosis (Kim *et al.*, 2002), while too much N can stimulate excessive vegetative growth that leads to plant deficiencies in other essential mineral nutrients (Majdi & Rosengrenbrinck, 1994). The excessive growth makes limbs more susceptible to breakage because the tissues are nutrient deficient, and thus, weaker (McCraw *et al.*, 2012). Excess N application can also delay fruit bearing and production (McCraw *et al.*, 2012). Young pecan trees are more susceptible to over-fertilization than older trees, so N fertilizer should be applied in small amounts several times throughout the growing season when establishing an orchard (Nesbitt *et al.*, 2010). There are also many environmental problems associated with excessive N application. Nitrogen is prone to leaching in wet soils and can contaminate surface and groundwater, which can lead to eutrophication and nitrate poisoning. It can also be converted to nitrous oxide (N_2O) and thus volatilized into the air as a gas, rendering it ineffective to plants and causing it to become an air pollutant. Worldwide nitrogen use efficiency, which is defined as plant productivity per unit of nutrient taken up, is only about 33%, so two-thirds of N applied to crops is unaccounted

for and therefore, believed to be in waterways, groundwater, and the atmosphere (Glass, 2003). The cost of N fertilizers, regardless of formulation, has greatly increased since 2008 and the application of several common N fertilizers has steadily increased for several decades (Economic Research Service, 2013). Therefore, over-fertilization is not only bad for the environment, but also very costly to pecan growers.

Another important factor in healthy tree growth is the establishment of a solid root system. Growth of roots formed after transplanting is vital for tree anchorage and survival since roots provide access to nutrients and water. The ability of roots to absorb N varies greatly depending on their size and age, as well as on soil characteristics like texture, aeration and drainage, and depth (Eissenstat & Yanai, 1997; Baddeley & Watson, 2004). Lifespan of fine roots varies between plant species and soil environmental conditions, but root diameter has the largest effect on root survivorship (Eissenstat & Yanai, 1997; Gaul *et al.*, 2009). The finest, outer-most roots generally have higher N concentrations and acquire more soil resources than roots of higher order (Pritchard *et al.*, 2008). However, because fine roots have a higher tissue N concentration and lower tissue density, they are more susceptible to decomposition (Pritchard *et al.*, 2008). Since fine roots most efficiently take up nutrients, it is important to identify periods during the growing season in which the greatest number of fine roots are produced and the greatest amount of root surface area is available for N uptake. Nitrogen uptake by fine roots rapidly decreases with time. Baddeley and Watson (2004) studied seasonal root production of *Prunus avium* and found that 50% of roots survived less than 100 days. A study by Volder *et al.* (2005) showed that, in grape, nitrate uptake

capacity of fine roots decreased by 50% within one day of root birth. This decrease in uptake effectiveness as the roots age is presumably the main reason for root turnover because root maintenance respiration has a high carbon (C) cost for the plant (Eissenstat & Yanai, 1997). Root turnover can be affected by several factors including leaf abscission and nutrient availability. Eissenstat and Yanai (1997) stated that plants attempt to maintain a C balance between roots and shoots. Since over-fertilization can lead to excess vegetative growth, it is assumed that to maintain the C balance, increased root growth would occur simultaneously, thus increasing production, and possibly turnover. Roots growing in soils with low nutrient content, however, have a longer lifespan because shedding roots can result in overall nutrient loss, so seedlings grown at low-to-very low N levels should have longer-living roots than those receiving adequate or too much fertilizer (Eissenstat & Yanai, 1997). However, there is conflicting literature that addresses the relationship between lifespan and N availability. In some studies, as lifespan shortens, root turnover generally increases. These studies have found that when there is more available N in soil, fine root turnover does indeed increase (Aber *et al.*, 1985; Nadelhoffer *et al.*, 1985). Others have found that root lifespan increases with available N in soil, thus decreasing root turnover (Keyes & Grier, 1981; Pregitzer *et al.*, 1993). Accurate timing of nutrient application is essential for nutrients like nitrate-N that are prone to leaching from the soil. If fertilizer applications were timed more effectively, less N would likely be lost into the environment, thus reducing pollution and the need to apply N at high rates.

1.2 Root detection methods

There are three standard methods commonly used for root detection: soil coring, minirhizotron, and trench profile mapping. There is ongoing debate over which type of root detection method is best, but it is difficult to compare the three methods in one study due to high rates of variation (Pierret *et al.*, 2005). Large variation between methods exists even when sampling is performed on the same experimental plots, which causes much confusion about fine root behavior and reinforces the need for more studies in this area (Hendricks *et al.*, 2006; Pritchard *et al.*, 2008). What follows is a review of the most common methods for root detection.

Coring. Sequential soil coring is the process of extracting soil cores at predetermined depths and removing fine roots from the soil through washing. This is the most common and cheapest method of root measurement (Jose *et al.*, 2001; Pierret *et al.*, 2005). Accuracy of soil coring is strongly dependent upon the sieve size used during washing. Large sieve sizes can result in loss of the finest roots, which can be a significant portion of total root mass. Sequential coring, when done properly, can give accurate numbers of standing mass and length, but provides no insight into fluxes of root production and/or root death that may have occurred during samplings dates. In-growth coring is similar to sequential coring in that it is removal of soil from the site followed by a washing process. The difference is that after cores are removed, the holes are filled with root-free soil surrounded by a selective mesh at approximately the same bulk density as surrounding soil (Hendricks *et al.*, 2006). This same portion, or core, is removed each time and re-filled with root-free soil. This allows researchers to measure

the growth of roots into the core for a known time interval to calculate production. If the time interval is kept short enough (e.g., < 2 months), mortality can also be observed this way by separating out live and dead roots. Problems with using coring to estimate root productivity lie in the assumption that sieved root-free soil does not affect root production or mortality compared to the original soil (Hendricks *et al.*, 2006), as the refilled soil material likely is not of similar compaction level as the bulk soil and removes competition from other roots. Another issue lies in the ability to separate out dead root material from live root material and organic matter. Many studies have shown that these processes occur simultaneously, so care must be taken to separate live and dead material when calculating turnover (Hendricks *et al.*, 2006).

Minirhizotron. Minirhizotron is one of the least invasive methods of root observation. Clear tubes are inserted belowground in close proximity to the plant being observed. There have been concerns about the effects of tube material on root growth and a study by Withington *et al.* (2003) showed that when comparing glass, acrylic, and butyrate tube materials, there was an effect on root lifespan and production, but the results appeared to be species specific, and thus, inconclusive. It can be assumed that if all tubes in the experiment are made of the same tube material, roots will be affected the same way, and comparisons between treatments will still be valid within the study. Tubes can be installed horizontally, vertically, or at an angle depending on the desired focus of the study. Angled tubes are common in field studies because of ease of installation and their use to observe vertical root distribution while minimizing water running down the outside of the tube. This preferential flow can create a different

microclimate around the tube surface and disrupt soil-tube contact (Johnson *et al.*, 2001). After tube installation, soil is left undisturbed for at least six months to allow soil to settle around the tube and for root production rates to return to normal (Joslin & Wolfe, 1999). When roots are disrupted, proliferation commonly occurs in the wounded area, so it is important to allow time for growth patterns to stabilize before image collection begins or install the tubes prior to the roots reaching that soil zone, e.g., by planting after tube installation (Joslin & Wolfe, 1999). Then, images are collected using a scanner or video recording device inside the tube. This method allows researchers to observe roots growing against the tube surface and collect information over time without disturbing the soil or disrupting root processes (Johnson *et al.*, 2001). Once images are collected, they are analyzed using software that tracks each root individually through time. Birth date, root diameter, total standing and individual root length, vigor (color), and date of death are commonly recorded (Pritchard *et al.*, 2008). These data are then used to determine seasonal production rates and fine root lifespan, and calculate turnover rates (Tierney & Fahey, 2002; Majdi *et al.*, 2007; Pritchard *et al.*, 2008). Minirhizotron studies yield the best results when conducted over a long period of time, usually several years, so that roots can be observed from birth to death across a range of seasons and environmental conditions. This method is based on the assumption that roots grow against the tube surface the same way they would grow in three-dimensional soil space. Minirhizotron also strongly relies on the ability of the observer to detect and trace fine roots during analysis.

Trench profile mapping. The trench profile mapping method can be used to observe roots in annual or perennial crops. It involves digging a trench or pit adjacent to the plants being studied. The pit surface is cleared and made even with a spade. A grid is set up on the trench wall, typically using nails, in blocks of 10-15 cm (Nemoto *et al.*, 1998; Achat *et al.*, 2008). Transparent sheets are used to record points at which roots intersect the grid. With this information, rooting patterns can be measured. The benefit of trench mapping is that both horizontal and vertical distribution of roots can be observed simultaneously (Logsdon & Allmaras, 1991). It also allows scientists to study soil characteristics and observe effects of soil on root growth and distribution (Achat *et al.*, 2008). Trenching can provide relative data pertaining to the number of roots present in a given area, but must include some type of destructive sampling or root extraction if root length densities are being measured (Kucke *et al.*, 1995; Achat *et al.*, 2008).

Ground penetrating radar. Another recent development in root observation is the use of ground penetrating radar. This technique has been used in archaeology to detect artifacts present below ground, but scientists have developed its capabilities to determine root biomass in areas where destructive methods are undesired. An antenna is positioned across the ground in direct contact with soil in a set grid pattern and moved along the grid at a predetermined pace. The antenna sends electromagnetic pulses into the soil and receives back reflections from buried objects, such as roots. These reflections create a parabola-shaped reading that is later interpreted through software. Although effective, this technique did not fit with the site conditions and research goals of this project. Soils with high moisture content and moderate to high clay content, such as those found at the

Texas A&M research orchard, greatly reduce the observable depth of the pulses and influence data output (Butnor *et al.*, 2001; Barton & Montagu, 2004). Also, the technique does not yet have the ability to determine diameter of roots less than 3 cm in diameter and cannot distinguish between live and dead roots (Butnor *et al.*, 2001), which were two of the main interests in this project. Additionally, although ground penetrating radar does work for determining a relative idea of root biomass in soil, it does not yet have the capability to observe roots as individuals through time.

Destructive harvest is a very important component of root studies, though it is not often done. There are several techniques used to excavate tree root systems. Often many of them are used during the course of an excavation. Roots can be uncovered manually by using hand tools and spades. This method is very labor- and time-intensive. Wet excavation involves flooding the soil with water, making the soil easier to remove. It reduces fine root breakage, but only works well in sandy textured soils (Danjon & Reubens, 2008). High-pressure air lances, or air spades, can also be used. The air spade blows soil away from roots with minimal damage to the roots themselves (Nadezhdina & Cermak, 2003). Soil is removed layer by layer, gradually revealing the root system underneath. Typically, a combination of these techniques is used. To harvest small trees, the air spade can be used to loosen soil to a depth of approximately 40 cm around the trunk, uncovering the center of the root system. The stem can then be pulled slowly upward with a mechanical digger while workers dig remaining roots out with hand tools. Once the entire root system is extracted, it can be cleaned with the air spade. Before the excavation process, it is important to expose a long surface root to measure *in situ* prior

to removal. This provides an estimate of the horizontal expansion of the root system. (Danjon & Reubens, 2008)

Practical limitations create a need for a sampling method to measure root system characteristics. There is no current standard sampling method; methodology is based on the objective of the study being conducted and the sample size needed. Thresholds are set based upon root diameter class. Once a threshold is set, roots excluded from the sample are pruned to eliminate them from the analysis. In fine root studies, for example, a threshold may consist of one randomly selected horizontal surface root and all of its connected branches. The branches would then be divided based upon diameter or branching order and then analyzed (Danjon & Reubens, 2008). In a study by Plourde *et al.* (2009), a grid was developed so the root system could be drawn to scale on paper using XYZ points. The drawing was scanned into a computer program that developed an outline of the root system's branching pattern and distribution. After roots were drawn and numbered, they were cut from the tree and taken to the lab for length and diameter measurements (Plourde *et al.*, 2009).

1.3 Summary

There is limited information currently available regarding the effects of N on pecan seedling growth and development, especially below ground. It is possible that current recommended N rates for young pecan seedlings can be lowered without detrimental effects to root growth. Conversely, less N fertilizer may stimulate roots to explore more soil space to search for nutrients, creating a stronger, more supportive root

system. In addition, large amounts of N could have a negative effect on root growth, much like it does on foliar growth. One study found that, although the reason is unknown, higher rates of N fertilizer harmed the growth of pecan seedlings (Conner, 2007). Pecan and several other horticultural crops grown in containers under high ammonium (NH_4^+) conditions had thinner, shorter, less branched, and darker roots than untreated control plants (Kim *et al.*, 2002). This reduction in root productivity is possibly due to a lowering of rhizosphere pH as a result of NH_4^+ application rather than a direct effect of NH_4^+ on root growth (Kim *et al.*, 2002). Kim *et al.* (2002) also concluded that N application as NH_4^+ strongly inhibits total biomass and root growth in container-grown pecan trees under laboratory conditions. Olsthoorn *et al.* (1991) also found that high rates of NH_4^+ decreased root length in Douglas Fir, leading to higher shoot: root ratios.

1.4 Objective and hypothesis

The objective of the proposed investigation was to observe, using the minirhizotron root observation technique, the effect of five N fertilizer rates (applied as ammonium sulfate) on root production, root lifespan and root turnover of young pecan seedlings grown in an orchard. The results were expected to be similar to those previously reported where high application rates reduced standing root length and root lifespan, while increasing root turnover. Root tracing analysis was then used to determine whether the expected decrease in standing root length was due to reduced production rates, increased mortality, or both. In addition, this study has yielded the first

detailed data describing seasonal patterns of root production of transplanted pecan seedlings in an orchard. These data are vital in determining correct fertilizer application amounts and times throughout the growing season.

CHAPTER II

MATERIALS AND METHODS

2.1 Experiment site

The experiment began in April 2009 and continued through June 2012 at the Texas A&M University pecan research orchard (lat. 30°31′N, long. 96°24′W, elevation 67 m), located west of College Station, TX. The orchard is located on Weswood silt loam soil (0 to 1% slope, fine-silty, mixed, superactive, thermic Udifluventic Haplustepts). Forty-two second-leaf, open-pollinated, bare-root ‘Mohawk’ seedlings were planted with a spacing of 3.8 x 15.2 m in early 2009. Trees were transplanted according to recommendations from Texas A&M AgriLife Extension Service. Holes 80-100 cm deep were dug and taproots pruned if necessary to sit firmly on the bottom of each hole. The crowns of the trunks were planted level with the surrounding soil surface, and soil was backfilled and packed tightly around the root systems. (McEachern, 2007) Throughout the course of the study, trees were micro-sprinkler irrigated every 1 to 2 weeks from May through October as needed. Tree rows were maintained vegetation-free using a glyphosate-based herbicide and alleys were left vegetated, primarily by bermudagrass, to reduce erosion and facilitate movement of heavy equipment during the growing season.

2.1 Fertilizer treatments

Fertilizer treatments were hand-applied as granular ammonium sulfate [$(NH_4)_2SO_4$] on 21 May and 21 June 2010, and 16 May and 13 June 2011. There were five N fertilizer treatments in the experiment, 0N, 0.25N, 0.5N, 1N, and 2N, where N= 229.5 kg ha^{-1}, the rate recommended by Texas A&M AgriLife Extension Service. There were eight replications for each treatment randomized throughout the experiment site (Table 1). Pre-weighed treatments were hand-distributed over a 1-m^2 area around each tree trunk.

Table 1 Treatment distribution at the experiment site. Each cell represents a pecan seedling subjected to one of the five N treatments, 0N, 0.25N, 0.5N, 1N, and 2N, where N= 229.5 kg ha^{-1}. G, guard tree; X, missing tree.

	Row 4	Row 5	Row 6	Row 7	Row 8	Row 9
Tree 1	G	G	G	G	G	G
Tree 2	0.25 N	0N	0N	0.5N	0.25 N	0N
Tree 3	1N	1N	0.5N	0N	0N	0N
Tree 4	0.25 N	0N	X	2N	1N	2N
Tree 5	0.5N	0.5N	0.25 N	2N	1N	2N
Tree 6	0.25 N	2N	X	0.25 N	1N	2N
Tree 7	0.5N	0.25 N	1N	1N	0.25 N	0.5N
Tree 8	1N	0.5N	2N	0N	0.5N	2N
Tree 9	G	G	G	G	G	G

In December 2012, i.e., 6 months after the collection of data ended, the area of soil between the two largest adjacent trees was excavated with an air spade to blow soil away from the coarse root system. The two trees were chosen from a randomly selected row in the orchard. Starting from the base of each tree, the air spade was used to excavate the main architectural roots between the trees to determine whether root system overlap occurred. The main horizontal roots in this space were exposed, photographed, and measured. The lateral roots were found to extend approximately 2.75 m, twice the width of the tree canopy. No root system overlap was found between the two adjacent trees, thus indicating root systems were not receiving neighboring treatments over the course of the experiment.

2.3 Minirhizotron

Twenty of the 40 trees were selected for the minirhizotron study based on trunk diameter and vigor after transplanting. Trees were divided into four diameter classes. One tree per diameter class was included in every treatment. A 1.8 m-long, 6.4 cm-wide (internal diameter) clear acrylic tube (CID Inc., Camas, WA) was installed next to each tree in April 2009. An auger was used to drill a hole for each tube at a 45° angle, 50 cm from the tree trunk. Consequently, the observable depth through each tube was approximately 110 cm. Tubes were placed in the holes and the holes backfilled with native soil. Each tube was capped at the bottom with a sealed rubber cap to prevent water from seeping in. White duct tape was wrapped around the top part of each tube that extruded above ground to prevent light penetration into the tube. The top end of the

tube was also covered by a removable black rubber cap. A foam rubber roll was placed in the first 40 cm of each tube to reduce temperature fluctuations along the length of the tube. Image collection began in February 2010, approximately one year after tube installation to allow disturbed soil to settle for maximum soil-tube contact. Images of standing root length were collected year-round, every two weeks with a CI-600 root scanner (CID Inc.) connected to a laptop computer to monitor root production and mortality. Digital images were 21 cm wide by 19.6 cm high and were taken at eight sequential depth increments in each tube, as shown in Fig. 1.

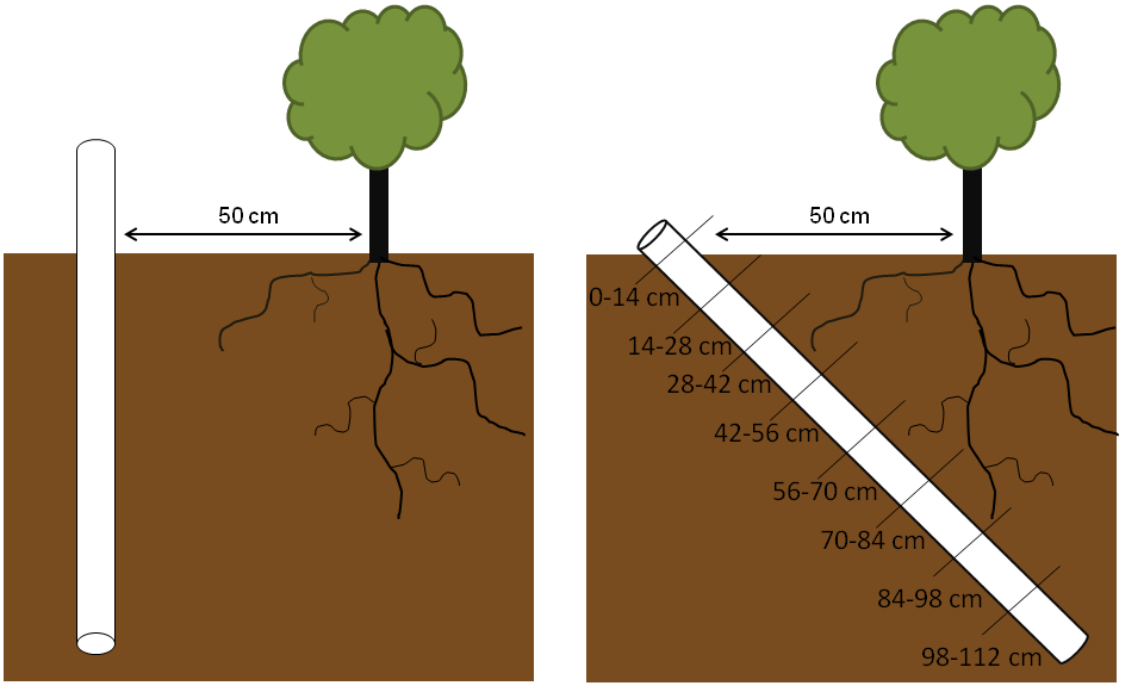

Fig. 1 Below-ground placement of minirhizotron tubes next to pecan seedlings receiving different rates of N fertilizers. Acrylic tubes were placed at a 45° angle parallel to the root system. Fine roots were observed at eight sequential depth increments for two growing seasons.

2.4 Environmental data

Weather data were collected using a weather station adjacent to the orchard. Soil moisture was collected once per week using TDR probes and a MiniTrase TDR reader (Soilmoisture Equipment Corp., Santa Barbara, CA).

2.5 Image analysis

Images from two of the eight depth increments, 14-28 cm and 98-112 cm, were processed using RootFly (Clemson University, Clemson, SC). Low quality images were enhanced for brightness and contrast before analysis to facilitate detection of roots. Each image was zoomed to five times magnification to trace fine roots. Individual root length and diameter were traced manually using a computer mouse in each image. Net root length production (RLP_{net}) was evaluated by subtracting root length death (RLD) from new root length production (RLP_{new}).

$$RLP_{net} = RLP_{new} - RLD$$

Standing root length (StRL was calculated by subtracting cumulative dead root length (C_{dead}) from cumulative new root length (C_{new}).

$$StRL = C_{new} - C_{dead}$$

Lifespan was calculated by subtracting date of root death from date of root appearance. Roots were considered dead upon the date of complete disappearance from the image. Roots that were still living at the conclusion of the study were censored, but still included in survival analyses. Life spans of living roots were calculated up to the last

day of the study. Those traced on the first date were excluded from the lifespan analysis because date of birth was unknown and occurred before image collection began. Roots were divided into three diameter classes, <0.5 mm, 0.5-1 mm, and >1 mm to determine differences in lifespan among roots of different diameters. Roots were also classified into one of three cohorts according to the date of appearance. Cohorts were early-season growth (March-June), late-season growth (July-October), and end-of season/dormancy (November-February). Turnover was calculated using the following formulas from Burton *et al.* (2000): T_3 was used for analysis because it was the most representative calculation of turnover rate, taking into account both root production and death.

$$T_1 = \frac{\text{average annual root length produced}}{\text{average standing root length}}$$

$$T_2 = \frac{\text{average annual root length death}}{\text{average standing root length}}$$

$$T_3 = \text{average of } T_1 \text{ and } T_2$$

2.6 Statistical analysis

The effects of N treatment depth (14-28 cm and 98-112 cm), and possible interactions of those, on standing root length were assessed using ANOVA. The data were grouped by season with each season analyzed separately, to decrease the number of repeated measures. The effects of N treatment, root diameter, depth, and birth season on the risk of root mortality for the duration of the study were estimated using Cox proportional hazards regression. Differences in root turnover rates were also determined using ANOVA. All statistical analyses were performed in JMP 11.0 (SAS Institute, Cary, NC, USA). All differences were considered significant at $P<0.05$.

CHAPTER III

RESULTS

3.1 Standing root length

At 14-28 cm, seedlings receiving the 1N treatment had the greatest standing root length during spring (P_{NS} <0.0001)(Fig. 2A). The 2N treatment showed decreased root length during the dormant season (P_{ND}<0.0001). At a soil depth of 98-112 cm (Fig. 2B), the 1N treatment still had the greatest average standing root length in spring (P_{NS} <0.0001), while 0N showed decreased root length in the dormant season (P_{ND}=0.0014). On average, the soil depth of 98-112 cm only contained 53% of the standing root length that occurred at 14-28 cm (means of 480.8 mm and 898.1 mm, respectively) (Fig. 2).

3.2 New root production

There was a significant depth effect on cumulative root length produced (P_{depth} <0.0001). Four times more root length was produced over the course of the study at 14-28 cm compared to 98-112 cm (Fig 3A). The amount of cumulative root death that occurred over the course of the study was similar to new root production and a strong depth effect was observed (Fig. 3B).

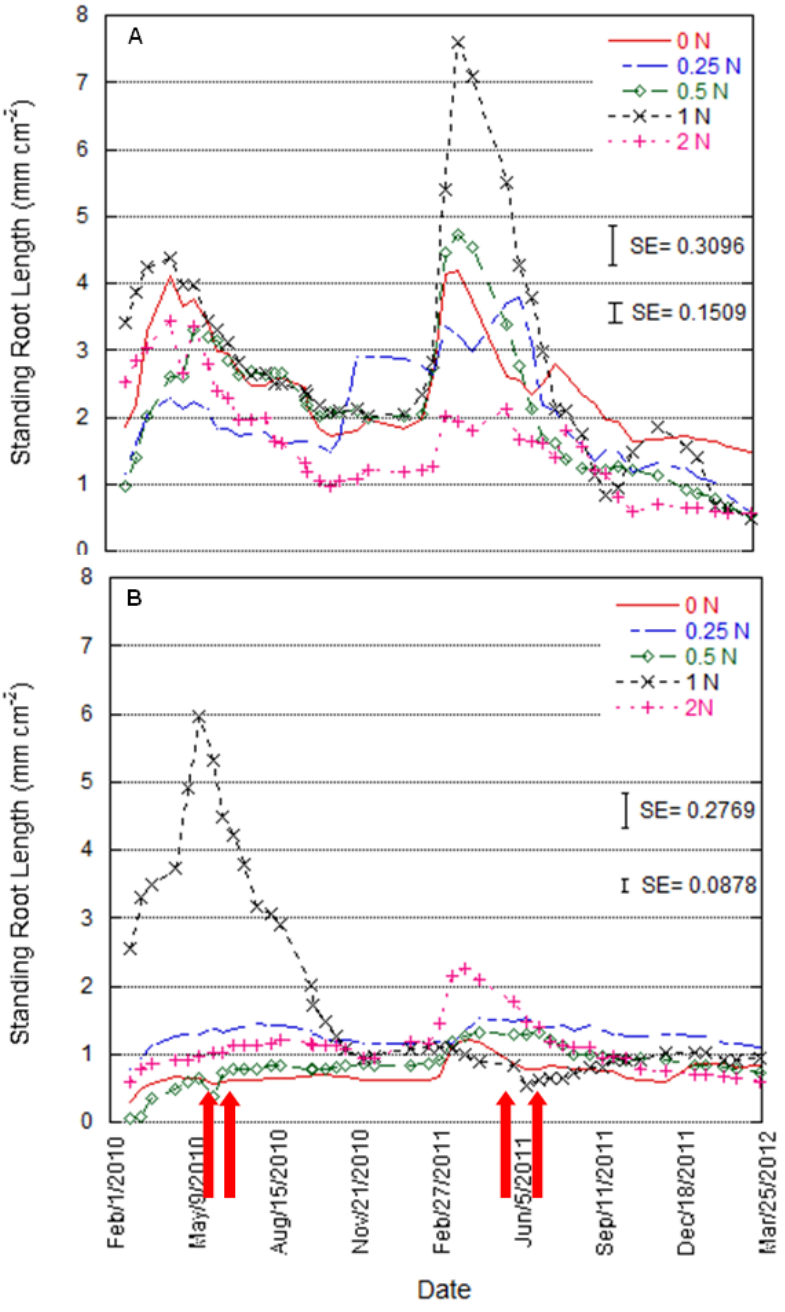

Fig. 2 Standing root length of pecan seedlings following four applications of five rates of N fertilizer. Application were conducted on 21 May and 21 June 2010, and 16 May and 13 June 2011) and fertilizer treatments (0N, 0.25N, 0.5N, 1N, and 2N, where N= 229.5 kg ha^{-1}, the rate recommended by Texas A&M AgriLife Extension Service) were hand-applied as granular ammonium sulfate [$(NH_4)_2SO_4$]. A) 14-28 cm soil depth; B) 98-112 cm soil depth. Standard error bars are for spring data (top) and dormant season data (bottom).

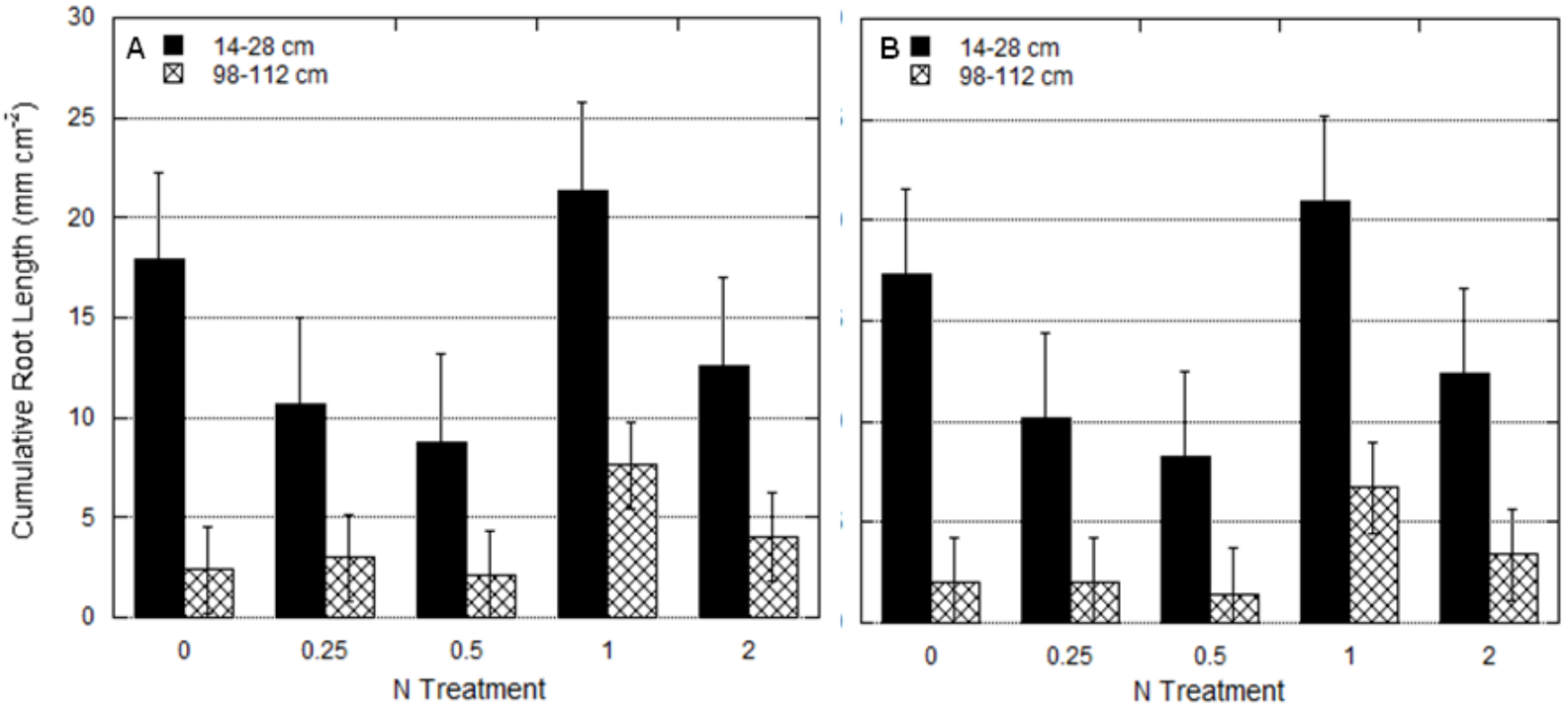

Fig. 3 Cumulative root production and root death. The total root length produced (A) and the total amount of root death (B) that occurred by pecan trees receiving one of five different N treatments at two soil depths after observation over two growing seasons.

Mean root diameter across all treatments and depths was 0.57 mm. The largest percentage of roots (59%) occurred in the 0.5-1.0 mm diameter range. Thirty eight percent of roots were classified as fine roots, i.e., less than 0.5 mm in diameter. Only 3% of roots traced were larger than 1 mm in diameter. There was a slight difference in distribution of diameter between depths. At 14-28 cm, 61% of roots produced were 0.5-1 mm and 36% of roots were less than 0.5 mm in diameter. At 98-112 cm, 52% of roots were 0.5-1 mm and 46% of roots produced were less than 0.5 mm in diameter. Most root production (61%) took place in the spring, as trees began to come out of dormancy into bud-break and the leafing out stage. Production declined severely, to 21%, in summer and early fall, and then declined even more in the winter months to just 16%.

3.3 Root mortality

At both soil depths, N treatment had a significant effect on lifespan ($P_N<0.0001$). Trees receiving the lower N rates (0.25N and 0.5N) had a lower risk of mortality and longer lifespan compared to the 0N treatment, while trees receiving higher N rates (1N and 2N), had a higher risk of mortality and shorter lifespan (Fig 4A and 4B). Soil depth also had a significant effect on root lifespan ($P_D <0.0001$). Roots located in deeper soil profiles lived significantly longer (131 d) than those located in more superficial soil profiles (Fig 4).

The effect of root diameter on risk of mortality was not consistent between depths. At 14-28 cm roots that were 1-2 mm in size had a lower risk of mortality than roots less than 1 mm in diameter ($P_{Diam} <0.0001$). There was a similar trend for root

mortality at 98-112 cm, however the effect was not statistically significant (Fig 4C and 4D).

Roots born in the spring (March-June) had a significantly higher risk of mortality than those born in summer, autumn, and winter (P_{Birth} <0.0001) (Fig 4E and 4F). Median lifespan for roots born in spring at a depth of 14-28 cm was 85 days, while median lifespan for roots born in summer and winter was 102 and 98 days, respectively. The same effect on lifespan was seen at 98-112 cm, where median lifespan was 186 d for spring-born roots, 323 d for summer-early fall, and 258 d for winter (P_{Birth} <0.0001).

3.4 Root turnover

Turnover was significantly affected by both N treatment and depth. When both depths were analyzed together, 2N had a higher turnover rate than 0.5N (P_N=0.0212). The rate of turnover was slower in deep soil profiles than in topsoil (P_D<0.0001).

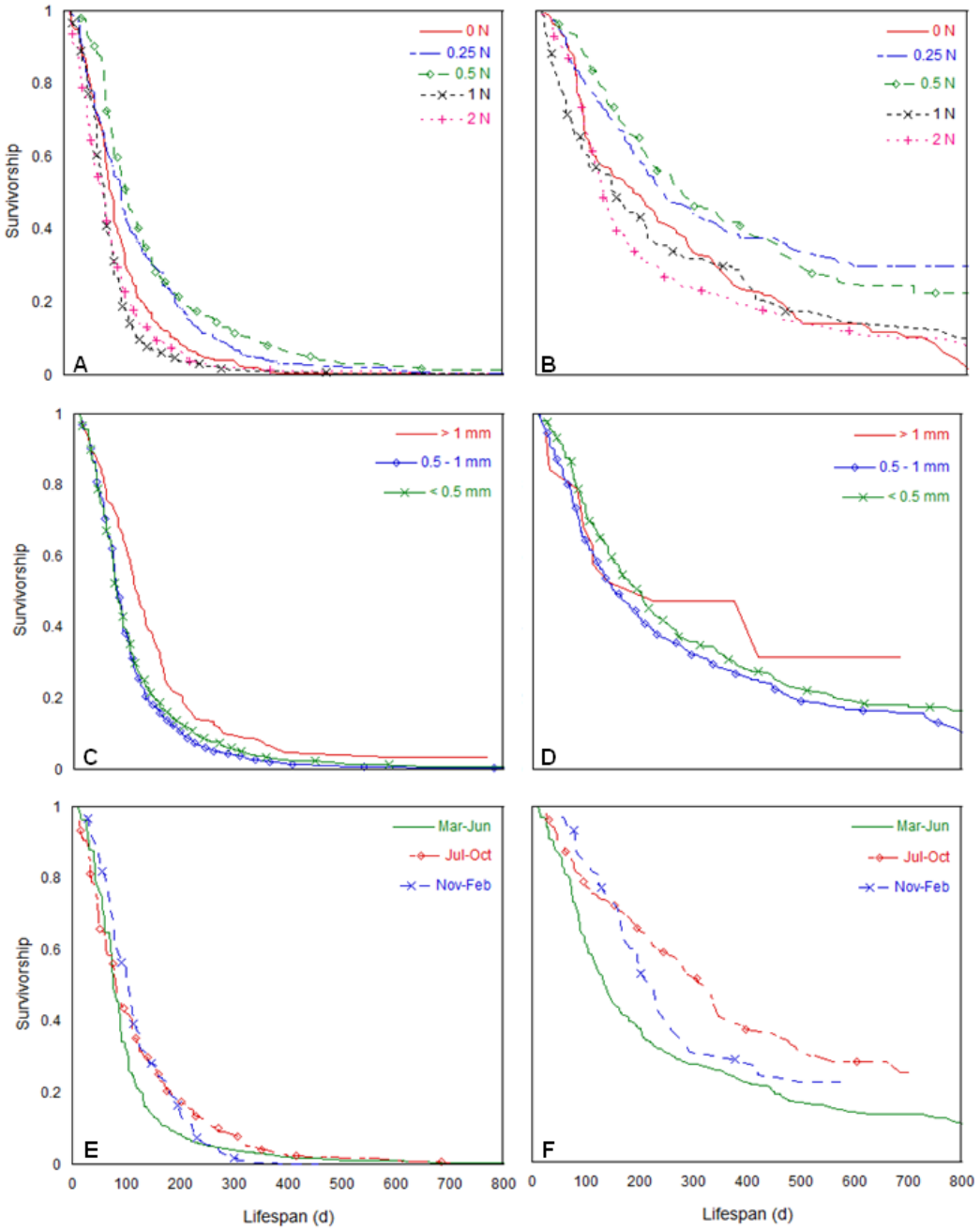

Fig. 4 Effects of N fertilizer rate (A, B), root diameter (C, D) and time of birth (E, F) on root survival in pecan seedlings following four applications of five rates of N fertilizer. Data refer to the 14-28 cm soil profile (A, C, E) and 98-112 cm soil profile (B, D, F).

CHAPTER IV

DISCUSSION

4.1 Standing root length

Trees receiving the 1N treatment had the greatest standing root length at 14-28 cm, as well as 98-112 cm. The 2N treatment showed decreased standing root length during the dormant season at 14-28 cm, while 0N showed decreased length during dormancy in deep soil. Kim *et al.* (2002) found similar results in a container seedling study on pecan, as roots receiving high rates of ammonium fertilizer were smaller in length and diameter, and showed decreased growth. These negative effects have been seen in several herbaceous species as well, and could be attributed to the decrease in rhizosphere pH after fertilizer application (Warncke & Barber, 1973) or inhibited uptake of other essential nutrients, such as Ca^{++} and Mg^{++} (Scoggins & Mills, 1998).

There were two peaks in standing root length, both of which corresponded with spring when trees began the leafing out stage that indicates the end of dormancy and the beginning of the growing season. The growth peaks were a result of seasonal growth patterns, not a response to fertilizer applications. The 0N, which did not receive fertilizer, exhibited growth peaks at the same time as other treatments and the peaks occur before the first fertilizer application in both years. Standing length decreased continually throughout the growing season and into the winter both years, with a slower decrease in 2010 than 2011. This could be due to environmental stress such as higher summer temperatures or differences in rainfall between the two years. Specific root

length (SRL) collected from soil cores over the same period showed varied results. In 2010, SRL increased through the year and peaked in October, while in 2011 SRL peaked in May and then decreased as expected through the growing season (Hannah, 2014). It is possible that in 2010 tree roots may not have reached equilibrium since being transplanted in 2009, and were more established in 2011. It could also indicate that pecan may have a species-specific tendency to produce a flush of roots late in the season, which would physiologically coincide with nut maturity and leaf drop. At 98-112 cm, the same increase in standing length was seen in 2011, but not in 2010. It is possible that since trees were transplanted, roots had not begun to penetrate soil that deep in large numbers yet. We had expected that high levels of N (2N) would decrease standing root length, while low to moderate levels would increase standing root length. The high fertilizer rate (2N) did respond as expected, while the lower rates did not. The 0.25N and 0.5N had lower standing root length on average than the 0N, which received no fertilizer.

4.2 New root production

The 1N treatment produced the highest amount of new root length over the duration of the study. We expected that 2N would have the lowest new root production, showing a negative effect of excess fertilizer, but that was not the case. We also expected that roots receiving lower N rates would respond favorably compared to roots receiving no fertilizer by showing production increasing as N increased, with the exception of the 2N. It is possible that with root growth present deep in the soil profile,

trees receiving 0N had access to N reserves below 1 m from previous years of agriculture production on the site and were not N-limited. A study by Jacobs *et al.* (2005) found that root growth response to fertilizer increased to a certain point (60 g N per seedling) and then began to decline. Smaller amounts of N applications have been shown to stimulate root growth, and we saw this in the 1N treatment, which had the greatest standing root length over the course of the study, but this was not observed in the lower rates (0.25 N and 0.5 N). Majdi and Andersson (2005) and Pregitzer *et al.* (1993) found that fine root biomass increased with added N fertilizer, while several other studies (Bloom *et al.*, 1985; Majdi & Persson, 1995) indicated that biomass decreased with added N fertilizer. In a container seedling study by Birk and Vitousek (1986), root:shoot ratios were lower in trees receiving fertilizer applications, so proliferation of root growth did not occur. Fertilized seedlings absorbed larger amounts of N, but growth per unit N was actually higher in N-limited plants. This indicates that even though plants were absorbing higher N levels, they were not utilizing it efficiently to increase growth, possibly due to other nutrients becoming limiting (Birk & Vitousek, 1986). It is also important to note that, even though standing root length fluctuated throughout the observation time, cumulative root growth produced and cumulative root death were similar within treatments, indicating that increased standing root length was a result of increased production, not decreased root death.

There was a very clear depth effect on root production. Almost twice as much root length was produced at 14-28 cm compared to the deep soil layer at 98-112 cm. Wu *et al.* (2013) found 87% of roots sampled from an alpine meadow in the upper 30 cm of

soil. In a review by Schenk and Jackson (2002), over 90% of the root profiles studied contained 50% of plant roots in the top 30 cm of soil. Our results reflected the findings of other research, and it was expected, as most root growth typically occurs where nutrients, moisture, and oxygen are more readily available.

In the group of roots recorded in this study, most had a diameter smaller than 1 mm. There was a shift toward a larger proportion of fine roots deeper in soil. This could be due to soil characteristics such as texture that may make soil less penetrable, or due to the role roots in deep soil play, which is unknown. Nagarajah (1987) found that soil texture strongly affects rooting depth in both 'Thompson seedless' and 'Ramsey' grape rootstocks. Roots in coarse textured soils grew deeply and had an even vertical distribution, while roots in finer soils were more concentrated in shallow soil layers and vertical distribution declined rapidly with soil depth (Nagarajah, 1987). Another possible explanation for this shift in fewer, smaller roots is oxygen (O_2) concentration in deep soil. Stolzy *et al.* (1961) and Grable and Siemer (1968) have demonstrated in snapdragon and corn that root elongation severely declines in response to limited O_2 concentration. The snapdragon study showed a 30% decrease in root growth in plants receiving low O_2 and rooting depth was restricted by as much as 50% (Stolzy *et al.*, 1961). Oxygen diffusion rates can be influenced by soil parameters such as bulk density and moisture content (Stolzy *et al.*, 1961).

We found that most new root growth occurred in the spring and early summer, which is also when seedlings come out of dormancy and undergo most of their vegetative aboveground growth. Production declined throughout the rest of the year and

very few roots were produced between November and February, while seedlings were dormant. New root production was less critical from November to February because fewer sinks were present in aboveground growth to drive the need to acquire nutrients. In addition, soil conditions were most likely not conducive to new root growth and survival at this time, as soil temperatures were below optimum conditions and trees were not receiving irrigation. Soil temperatures observed in the same region of Texas showed fluctuated between 10 and 30°C from winter to mid-summer (National Water and Climate Center, 2014). In a study of black walnut (*Juglans nigra* L.), root growth was inhibited at soil temperatures of 10°C, but growth rate reached its peak at 17-19°C, while root number peaked at 21°C, which was the highest temperature observed during the study (Kuhns *et al.*, 1985). Other studies have also found that time of birth can influence mortality in some species (Anderson *et al.*, 2003; Gu *et al.*, 2011; Adams *et al.*, 2013).

4.3 Root mortality

In our study, roots of trees receiving low N rates (0.25N and 0.5N) had a significantly longer root lifespan than the unfertilized trees, while those receiving high N rates (1N and 2N) had a significantly shorter lifespan compared to the unfertilized trees. These results showed the increased mortality risk with increased N application that we were expecting. The high N and recommended rates did decrease lifespan, while small amounts of N had the opposite effect. There are many conflicting studies regarding the effects of N treatment on root lifespan. Studies have shown that application of N in patches increased root lifespan compared to roots receiving no treatment or treatment of

water only (Pregitzer *et al.*, 1993; Eissenstat & Yanai, 1997). Adams *et al.* (2013) found that lifespan increased with the application of fertilizer in fine-rooted species, but the response was not significant in coarse-rooted species included in the study. Plants have the ability, to some extent, to control root lifespan and selectively cause root tissue to die off when the tissue is no longer beneficial through a process called shedding. Chen and Brassard (2013) stated that in nutrient-limited soils fine root lifespan should be longer because plants should retain many of their fine roots to conserve C instead of shedding the older, less active roots to produce new ones, but their results showed no significant effect of fertilization on root lifespan. It is possible that trees receiving little to no N fertilizer were retaining roots to conserve C, whereas those receiving adequate amounts of N had the ability to shed roots more quickly because the nutrients held within those roots were not limiting to plant growth.

Roots growing in deeper soils had a much lower risk of mortality and lived on average 131 d longer than those in upper soil layers. Other studies have shown similar results. In 'Concord' grape, roots growing in deeper soils consistently had a lower mortality risk (Anderson *et al.*, 2003); another study in grape attributed lower mortality risk to very different moisture conditions in the subsoil (Comas *et al.*, 2010). There could be several possible reasons roots live longer in deeper soils, including decreased herbivory and more stable soil conditions (Wells *et al.*, 2002). In sugar maple (*Acer saccharum* Marsh.) root respiration was greatly reduced in deep soil profiles (40-50 cm) demonstrating that roots in deep soils are less metabolically active due to low O_2 and increased CO_2 concentrations and may have functions other than nutrient uptake

(Pregitzer *et al.*, 1998). Eissenstat and Yanai (1997) also stated that fine roots also serve as a source of meristematic tissue in the soil, waiting to initiate root growth when water and/or nutrients become available. Respiration is a significant cost of root maintenance, so lower respiration rates reduce C demand on the plant. Since these roots are not as costly to maintain, this could explain greater root longevity in deep soil.

Lifespan was inversely correlated with root diameter, as risk of mortality increased with decreasing diameter in shallow soils. In deeper soils, the effect followed the same general pattern of smaller diameter roots having increased risk of mortality, but the results were not statistically significant. Finer roots are shown to have a lower C:N ratio than roots of larger diameter, so their tissues have higher concentrations of N, making them more susceptible to herbivory and decomposition by microorganisms (Eissenstat & Yanai, 1997; Huang *et al.*, 2010).

Roots born in spring had a higher mortality risk than those born at any other point during the year. Other studies have also found that timing of root birth has an effect on root lifespan. Spring-born roots of 'Concord' grape also had a shorter lifespan than those born at other times in the growing season (Anderson *et al.*, 2003). This could be attributed to the presence of more soil moisture and nutrient availability and increased microbial activity in soil during spring. With increased nutrient availability, roots absorb more nutrients from the soil matrix, thus increasing the concentration of nutrients within root tissues. Fine roots born in spring would be more susceptible to decomposition than roots born at other times with fewer available nutrients in soil that are likely taking up less nutrients. Soil microorganisms have been shown to be more metabolically active

during warmer times of year (Blume *et al.*, 2002). Depending on the function of these microorganisms, they could contribute to increased nutrient availability or decomposition of fine roots, or both. Hishi and Takeda (2005) conducted a root life cycle study of Japanese cypress (*Chamaecyparis obtusa* Sieb et. Zucc.) and found that functions of roots were different depending on the time of birth. Those born at 0-4 months, or the beginning of the growing season, were colonizers responsible for introducing new root tips into the soil, roots from 4-7 months were responsible for branching and creating root clusters, and those from 7-12 months maintained the presence of root clusters even as ephemeral roots began to die (Hishi & Takeda, 2005). Spring-born roots may have a different function, such as colonizing new soil, which could explain their shorter lifespan compared to roots that may maintain root growth that has already been established.

4.4 Root turnover

Root turnover followed the same general pattern as production in our study. We would expect as the amount of root length produced increases and lifespan decreases, that turnover rate would increase. Typically, turnover rates increase as N availability increases (Aber *et al.*, 1985; Majdi & Andersson, 2005). The results of the lower N applications (0.25N and 0.5N) in this experiment did not agree with results reported in the literature by Aber *et al*. (1985) or Majdi & Andersson (2005), as there was a decline in turnover when N was applied in small amounts. There is a possibility that trees limited nutrient loss by retaining root tissues that contained nutrients in those low N

environments (West *et al.*, 2004). Mycorrhizal associations should also be considered, as pecan has been shown to develop ectomycorrhizal associations with several species of fungi (Bonito *et al.*, 2011). Even though no mycorrhizal growth was observed during image analysis, associations may have been present in other parts of the root system. Mycorrhizae have also been shown to increase root lifespan in some species (Atkinson *et al.*, 2003). An increase in lifespan would thus lead to a slower turnover rate. In our experiment, it is a possibility that trees receiving low amounts of fertilizer had a decreased turnover rate because they had formed these associations and the plant was maintaining associations by keeping those roots alive to sustain the fungi. Since the 0N had a greater production and turnover rate than the two low N treatments, those trees may have been receiving more benefits from the mycorrhizal associations than those trees that were fertilized. Increases in available N have been shown to decrease the number of mycorrhizal roots (Majdi *et al.*, 2001).

CHAPTER V

CONCLUSION

There is much conflicting literature on the effects of N on root growth. It could be that nutrient responses are species-specific. It is also possible, and quite likely, that there is an intricate balance between the C cost for maintaining a root versus the benefits of nutrient acquisition (Sibly *et al.*, 1986). Since trees receiving more N produced more root growth in deep soils, it is possible that those fine roots were exploring soil for other, more limiting nutrients such as P or micronutrients such as Zn and Fe that are less mobile (Eissenstat *et al.*, 2001). High rates of N decreased root production, while also decreasing lifespan. Low N rates were shown to increase lifespan in this study compared to the unfertilized and high N rates. The increase in lifespan under low N conditions could be attributed to mycorrhizal fungi or to a plant response to maintain tissue to reduce nutrient loss in an already-limiting soil environment. Root diameter had a significant effect on mortality, and the risk of root death increased as root diameter decreased. Root growth did occur in both depths observed, but significantly more root growth occurred in shallower soils compared to deep soils, which was expected. Soil depth also played a role in root mortality. Roots in deeper soil lived significantly longer than those closer to the surface. Most of the roots produced in the study appeared in the spring (March-June) and had a shorter lifespan than those born in late summer, fall, or winter.

The data collected in this study support current Extension recommendations that 229.5 kg ha^{-1} N is the correct rate for application to pecan seedlings. Recommendations also state that fertilizer application should take place in spring with split applications from April-July. Since we observed most new root growth in spring and early summer (March-June), pecan could benefit from fertilizer applications beginning a few weeks earlier in March to fully coincide with maximum root growth, and thus reach maximum uptake. More field trials should be conducted in other growing regions to support these findings. It is important to note that long-term effects of root system size and mortality on yield and overall tree health are unknown in pecan. Future studies on the impact of root system size and productivity on yield and tree health would be beneficial and an important part of understanding how seedling establishment can affect long-term productivity in transplanted fruit and nut trees.

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APPENDIX A

RISK RATIO TABLES FOR ROOT MORTALITY

Table 2 Proportional hazards analysis of root lifespan for 14-28 cm and 98-112 cm. Treatment received and soil depth both impacted root survival in pecan.

Depth	14-28 cm			98-112 cm		
Treatments	25% failure (d)	50% failure (d)	75% failure (d)	25% failure (d)	50% failure (d)	75% failure(d)
0 N	56	89	128	84	180	364
0.25 N	56	105	181	119	238	.
0.5 N	75	114	190	133	271	617
1 N	50	74	100	56	148	393
2 N	42	72	106	82	125	268

Table 3 Risk ratios of roots by depth and N treatment. High rates of N increased root mortality risk, while soil depth decreased the risk of root mortality (P_N <0.0001*, <0.0001*) ($P_{Nxdepth}$= 0.0088*).

Depth	Treatment	Treatment	Risk Ratio	*P*	Lower 95%	Upper 95%
14-28 cm	0.25 N	0 N	0.7564	<0.0001*	0.6808	0.8397
	0.5 N	0 N	0.5837	<0.0001*	0.5188	0.6558
	0.5 N	0.25 N	0.7717	<0.0001*	0.6805	0.8746
	1 N	0 N	1.3043	<0.0001*	1.1919	1.4274
	1 N	0.25 N	1.7244	<0.0001*	1.5551	1.9138
	1 N	0.5 N	2.2344	<0.0001*	1.9942	2.5076
	2 N	0 N	1.2696	<0.0001*	1.1456	1.406
	2 N	0.25 N	1.6785	<0.0001*	1.4948	1.885
	2 N	0.5 N	2.1749	<0.0001*	1.9188	2.4673
	2 N	1 N	0.9734	0.5993	0.8797	1.0761
98-112 cm	0.25 N	0 N	0.5999	<0.0001*	0.4635	0.7744
	0.5 N	0 N	0.6285	0.0006*	0.4797	0.8198
	0.5 N	0.25 N	1.0476	0.7447	0.7909	1.3851
	1 N	0 N	1.0339	0.7715	0.8263	1.2965
	1 N	0.25 N	1.7233	<0.0001*	1.3559	2.2012
	1 N	0.5 N	1.6450	<0.0001*	1.2816	2.1258
	2 N	0 N	1.1959	0.1122	0.9592	1.4952
	2 N	0.25 N	1.9933	<0.0001*	1.5736	2.5389
	2 N	0.5 N	1.9028	<0.0001*	1.4873	2.4522
	2 N	1 N	1.1567	0.1607	0.9438	1.4184

Table 4 Risk of pecan root mortality based on diameter class. Fine roots had a higher risk of mortality than roots of larger diameter. ($P_{diameter}$ <0.0001*, 0.0701).

Depth	Diameter	Diameter	Risk Ratio	*P*	Lower 95%	Upper 95%
14-28 cm	>1 mm	<0.5 mm	0.6229	<0.0001*	0.5141	0.7477
	>1 mm	0.5-1 mm	0.6506	<0.0001*	1.2856	1.8561
	<0.5 mm	0.5-1 mm	1.0444	0.2291	0.9729	1.1206
98-112 cm	>1 mm	<0.5 mm	0.9099	0.7591	0.4645	1.5979
	>1 mm	0.5-1 mm	0.7676	0.3738	0.3935	1.3398
	<0.5 mm	0.5-1 mm	0.8437	0.0273*	0.7249	0.9812

Table 5 Season of root birth has an effect on the risk of root mortality in pecan seedlings. Those born from Mar-June have a higher risk of mortality than those born at other times during the year. (P_{birth} <0.0001*, <0.0001*).

Depth	Birth Season	Birth Season	Risk Ratio	*P*	Lower 95%	Upper 95%
14-28 cm	July-Oct	Mar-June	0.8303	<.0001*	0.7626	0.9029
	Nov-Feb	Mar-June	0.7623	<.0001*	0.6945	0.8367
	Nov-Feb	July-Oct	0.9188	0.1313	0.8228	1.0256
98-112 cm	July-Oct	Mar-June	0.5369	<.0001*	0.4416	0.6484
	Nov-Feb	Mar-June	0.5935	0.0001*	0.4408	0.7824
	Nov-Feb	July-Oct	1.1054	0.5429	0.7959	1.5132